

# Synergistic effects of $\alpha$ -pinene and **exo**-brevicommin on pine bark beetles and associated insects in Arizona

R. W. Hofstetter<sup>1</sup>, Z. Chen<sup>1</sup>, M. L. Gaylord<sup>1</sup>, J. D. McMillin<sup>2</sup> & M. R. Wagner<sup>1</sup>

<sup>1</sup> School of Forestry, Northern Arizona University, Flagstaff, AZ, USA

<sup>2</sup> USDA Forest Service, Forest Health Protection, Flagstaff, AZ, USA

## Keywords

*Dendroctonus brevicomis*, *Dendroctonus frontalis*, *Elacatis*, *Enoderus*, *Temnochila*, bark beetle, lure, pheromone, *Pinus ponderosa*

## Correspondence

Richard W. Hofstetter (corresponding author),  
School of Forestry, Northern Arizona  
University, Box 5018, Flagstaff, AZ 86011-  
5018, USA. E-mail: rich.hofstetter@nau.edu

Received: September 5, 2007; accepted:  
November 15, 2007.

doi: 10.1111/j.1439-0418.2007.01263.x

## Abstract

The southern pine beetle (*Dendroctonus frontalis*) and western pine beetle (*Dendroctonus brevicomis*) cause significant mortality to pines in the southern and western United States. The effectiveness of commercial lures at capturing these bark beetles in Arizona has not been tested and may vary from other regions of their distribution. We conducted experiments using baited Lindgren funnel traps to investigate (i) if *D. frontalis* is more attracted to the standard commercial lure for *D. brevicomis* (frontalin + *exo*-brevicommin + myrcene) than the *D. frontalis* lure (fronta-

## Introduction

Bark beetles (Coleoptera: Curculionidae, Scolytinae) use pheromones and plant compounds to focus beetle attacks on host trees (i.e. mass aggregation; Wood 1973, 1982a,b). For species that colonize living trees, it is important to cooperate with conspecifics to overcome tree defensive mechanisms (Raffa 2001 and references within). When two or more species

colonize the same tree, species-specific pheromones may promote successful multi-species aggregations (Svihra et al. 1980; Smith et al. 1990) and serve to partition the resource and minimize interspecific competition (Lanier and Wood 1975; Light et al. 1983; Rankin and Borden 1991). Cross-attraction by species successfully colonizing living trees infers a potential mutualism(s), while strong deterrence of pheromones of heterospecific beetles suggests an

antagonistic relation(s) between species. In the genus *Dendroctonus*, female beetles initiate attack, excavate galleries under the bark, and release aggregation pheromones that are attractive to both sexes (Borden et al. 1986; Raffa et al. 1993). Male beetles seek females within trees and produce pheromones which further facilitate colonization of the host tree (Raffa et al. 1993). These pheromones are generally species specific, but there are usually chemical components of the pheromones that overlap (Byers 1989).

In Arizona, USA, two aggressive bark beetles species, *Dendroctonus frontalis* Zimmerman and *Dendroctonus brevicomis* LeConte are sympatric, cohabit the same trees (Breece et al. in press), and show similar seasonal patterns in abundance within ponderosa pine (*Pinus ponderosa* Dougl. ex. Laws.) forests (Sanchez-Martinez and Wagner 2002; Gaylord et al. 2006; Williams et al. in press). Since 2000, these species have caused significant mortality to ponderosa pine forests (USDA Forest Service 2001–2006). Previous trapping efforts for these species in northern Arizona have used only the Western Pine Beetle (WPB) lure (*exo*-brevicommin, frontalin and myrcene) or the Southern Pine Beetle (SPB) lure without a terpene component (only frontalin) (Sanchez-Martinez and Wagner 2002; Gaylord et al. 2006; Williams et al. in press). In the southeast United States where *D. brevicomis* does not occur, *exo*-brevicommin has been shown to reduce or have no affect on *D. frontalis* captures within beetle infestations (Payne et al. 1977). However, recent studies by (Pureswaran et al. in press) have found that the presence of *exo*-brevicommin significantly increase trap capture in Mississippi. In Arizona,

where *D. frontalis* and *D. brevicomis* occur in sympatry, relative attraction to *exo*-brevicommin by *D. frontalis* in the presence of terpenes is unknown.

Commercially produced pheromones used for beetle-monitoring are generally species specific but are not regionally tested (Byers 2004) and, therefore, may not accurately assess population size and the relative abundances of species (Billings and Bryant 1983). Most commercially available lures result from studies that take place in only a few locations throughout the beetle's distribution. For instance, studies in California and Oregon suggest that *D. brevicomis* uses the terpene myrcene to synergize its pheromone (Bedard et al. 1969; Pitman and Vite 1971; Sturgeon 1979; Byers 1982). These studies have contributed to the production of the WPB lure which uses the monoterpene myrcene, as a synergist, with beetle-produced pheromones – *exo*-brevicommin and frontalin. However, the considerable geographical variation in monoterpene profiles of host trees across its geographical range (Smith 2000) may influence the host location behaviour of *D. brevicomis* (Sturgeon 1979) and *D. frontalis*, as well as other bark beetles and associated insects (Wood 1982a,b; Raffa 2001). For instance, while the monoterpene component of ponderosa pine in Arizona, USA is extremely variable, in general,  $\alpha$ -pinene is the predominant component while myrcene comprises very little of the resin of these trees (table 1). In contrast,  $\alpha$ -pinene in California is a minor monoterpene component (table 1; Smith 1975). Geographical differences in bark beetle response to pheromone lures can create a problem in optimizing lures for pest management (Berisford et al. 1990;

**Table 1** Chemical components of terpene blend used in lures during Experiment 2, and monoterpene composition of resin from 64 ponderosa pines in the Coconino N.F., Arizona compared with resin composition of ponderosa in Sierra Nevada California and loblolly pine (*P. taeda*) in Mississippi. Terpene blend created by ChemTica Internacional, S.A., Costa Rica

Terpenes	Terpene blend lure (% comp.)	Arizona (% mean $\pm$ STD)	California <sup>1</sup> (% mean $\pm$ STD)	Mississippi <sup>2</sup> (% mean)
$\alpha$ -pinene	45	46 $\pm$ 15	1	72 $\pm$ 10
$\beta$ -pinene	15	7 $\pm$ 13	50	20 $\pm$ 13
3-carene	24	31 $\pm$ 13	30	0
Myrcene	12	3 $\pm$ 2	3	3 $\pm$ 2
Terpinolene	4	2 $\pm$ 1	?	?
Limonene	0	7 $\pm$ 6	10	3 $\pm$ 3
$\beta$ -cubebene	0	3 $\pm$ 3	?	?
Borneol acetate	0	1 $\pm$ 1	0	1 $\pm$ 1
Others			6	1 $\pm$ 1
	100	100	100	100

Arizona monoterpene analyses by J. Mahfouz, USDA Forest Service, Southern Research Station (unpublished).

<sup>1</sup>Resin composition of ponderosa pine from Sierra Nevada near Placerville, CA (Smith 1975).

<sup>2</sup>Resin composition of loblolly pine from Mississippi at Harrison Experimental Forest, Saucier, MS (Strom et al. 2002).

Skillen et al. 1997; Aukema and Raffa 2005) and minimize predator captures within traps (Hermes et al. 1991; Raffa and Dahlgren 1995; Aukema et al. 2000; Seybold et al. 2006).

We conducted two field trapping experiments to investigate the attractiveness of current commercially available pheromone lures and lures with modified host volatiles for *D. frontalis*, *D. brevicomis* and their associated insects in Arizona. Objectives for the first experiment were to determine if *D. frontalis* is attracted to lures with *exo*-brevicomin (WPB lure), and whether the replacement of myrcene with  $\alpha$ -pinene changes trap catches.  $\alpha$ -Pinene is the primary monoterpene component used to attract *D. frontalis* in the southeast United States (Billings and Bryant 1983). The objective for the second experiment was to determine whether capture rates and lure preferences vary across pine forests throughout Arizona. For this experiment, we added an additional lure, frontalin + *exo*-brevicomin + terpene blend (modified synthetic re-creation of the terpene component of Arizona ponderosa pine, table 1) and compared its attraction to beetles and associated insects to the WPB lure with myrcene or  $\alpha$ -pinene. Improved lures for *D. brevicomis* and *D. frontalis* will allow for more effective monitoring of these species throughout this region and may lead to a separate lure for each species. Disparities among predators and bark beetles could select for particular lures for biological control. The results of these studies also provide insight into the aggregation behavior and competitive interactions of sympatric bark beetles species.

## Materials and Methods

### Experiment 1 – comparison of commercial lures and terpene alternatives

Trapping Experiment 1 was conducted to compare the relative attractiveness of commercially available pheromone lures and lures with modified components to *D. frontalis*, *D. brevicomis*, and associated insects in ponderosa pine stands within the Coconino National Forest of north-central Arizona. Ten blocks of seven 12-unit funnel traps (Phero Tech Inc., Delta, BC, Canada; Lindgren 1983) were arranged in a circular pattern, with 50 m between adjacent traps. Blocks were separated by a minimum of 500 m and located within 200 km of Flagstaff, Arizona (latitude 35°10'N, longitude 111°45'E, elevation 2080 m). Each trap was hung on a metal conduit at least 3 m from the nearest

tree with bottoms of traps 1 m above the ground. Each trap was randomly allocated to one of seven treatments and its location re-randomized each week. Lures were attached to the middle funnel of the trap. A 3 × 3 cm section of Spectracide® Bug Stop® pest strip (18.6% Dichlorvos, United Industries Corp., St. Louis, MO, USA) was placed into each collection cup to kill captured insects and reduce predation by predatory insects. Trap catches were collected weekly for 6 weeks from May 30, 2005 to July 5, 2005. This design resulted in  $n = 10$  blocks × 6 sample periods = 60 collections for each of the seven treatments. All Coleoptera, except Buprestidae, Cerambycidae and Elateridae, were identified to species or genus.

The following treatments were tested to determine their attractiveness to *D. frontalis*, *D. brevicomis* and associated insects: (i) blank control (no lure), (ii) frontalin, (iii) frontalin + myrcene, (iv) frontalin +  $\alpha$ -pinene [25% (–)  $\alpha$ -pinene + 75% (+)  $\alpha$ -pinene], (v) *exo*-brevicomin + frontalin + myrcene, (vi) *exo*-brevicomin + frontalin +  $\alpha$ -pinene [25% (–) + 75% (+)], and (vii) *exo*-brevicomin +  $\alpha$ -pinene [25% (–) + 75% (+)]. Frontalin was released at a rate of  $5.0 \pm 0.5$  mg/day, myrcene at  $22.0 \pm 1.0$  mg/day, and *exo*-brevicomin at  $4.0 \pm 0.6$  mg/day with polyethylene bottles under field conditions (products from Phero Tech Inc., Delta, British Columbia; three-component WPB lure). The  $\alpha$ -pinene (Sigma-Aldrich, St. Louis, MO, USA) mixture was released from polyethylene bottles at a rate of  $9.0 \pm 0.8$  mg/day in the field.

Analysis of variance was performed on each insect species for which more than five individuals were captured per trapping period. Data were analysed using a randomized complete block design (SAS PROC MIXED; SAS 1991–2000). Block and Block by Treatment interaction were considered random effects. In some cases, we tested combinations of predator or competitor species that showed significant treatment effects. Data were square-root transformed before analysis to reduce heteroscedasticity, although tables and figures show raw means and standard errors. Where significant treatment effects occurred ( $\alpha \leq 0.05$ ), differences were compared using pairwise *t*-tests on least squared means (Carmer and Swanson 1973).

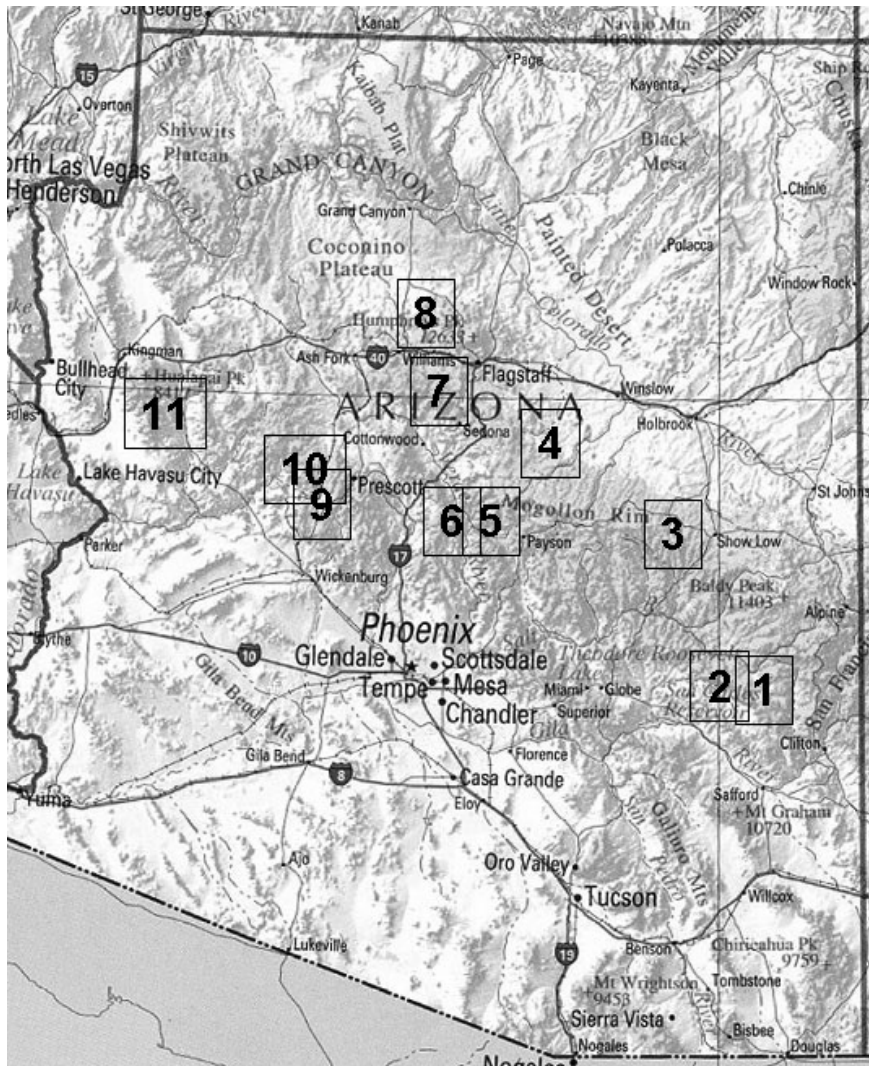
### Experiment 2 – response to terpenes across Arizona

Trapping Experiment 2 was conducted to test the relative attractiveness of different terpenes (myrcene,  $\alpha$ -pinene and a terpene blend) to *D. frontalis*,

*D. brevicomis* and associated insects across a broader geographical range and variation in oleoresin of ponderosa pine. Traps were located at 11 locations in ponderosa pine stands within National Forests across Arizona (fig. 1) between 1825 and 2235 m elevation. Three lures were tested (*exo*-brevicommin + frontalin + myrcene,  $\alpha$ -pinene and terpene blend) at each location. Release rate of each component compound was similar to Experiment 1. The terpene blend was dispensed from 1.8 ml polyethylene bottles with a release rate of  $12 \pm 0.5$  mg/day during the course of the study. Each lure was attached to a 12-unit funnel trap placed 30 m apart. All locations were separated by >5 km. Trap set up and use of the pest strip (insecticide) was similar to Experiment 1. Trap catches were collected after 1–2 weeks between June 15, 2006 and August 15, 2006. All insects were identified to species or genus. For some trap locations and dates, we were unable to identify either sex or

species of a proportion of *Dendroctonus* specimens due to poor condition of the insects within traps, and for these collections, tests of *Dendroctonus* species- and sex-ratios were not performed.

The terpene blend component of the lure (table 1) was a modified restitution of the monoterpenes found in resin of ponderosa pine located in the Coconino National Forest of Arizona. Constitutive resin was collected from 64 trees distributed across 130 km<sup>2</sup> of ponderosa pine-dominated forest during June 2005. The monoterpene component of the resin from each tree was analysed using a 6890 Gas Chromatographer equipped with a 5973 MS (Hewlett-Packard Corp., Palo Alto, CA, USA) with a HP-5MS 30 m length  $\times$  250  $\mu$ m ID  $\times$  0.25  $\mu$ m thickness column. The temperature programme was 60°C for 1 min, 6°C/min to 200°C, then 15°C/min to 250°C. Flow rate was 0.9 ml/min and the injector temperature was 200°C. We identified compounds



**Fig. 1** Locations of trap sites used to investigate bark beetle and predator response to pheromones and host plant terpenes. Locations: (1) Apache-Sitgreaves N.F., site 1 (33°13'N, 109°21'W; elevation: 1875 m), (2) Apache-Sitgreaves N.F., site 2 (33°22'N, 109°22'W; elevation: 2149 m), (3) Fool Hollow State Park (34°16'N, 110°04'W; 1899 m), (4) Mormon Lake (SW of Flagstaff; 35°10'N, 111°45'W, 2235 m), (5) Tonto N.F., site 1 (NW of Flagstaff, 35°23'N, 111°57'W, 2179 m), (6) Tonto N.F., site 2 (SE of Flagstaff; 34°53', 111°25'W, 2209 m) (7) Centennial Forest (N 34°27'N, 111°27'W, 2122 m), (8) Kendrick Mt. (N 34°28'N, 111°27'W, 2139 m), (9) Prescott N.F., site 1 (34°32'N, 112°33'W, 1848 m), (10) Prescott N.F., site 2 (34°35'N, 112°33'W, 1867 m), and (11) Hualapai, AZ (35.089° N, 113.868° W, 1825 m).

by their mass spectra and matched the retention time with known standards. Resin samples were dissolved in pentane with a *p*-cymene internal standard. The final resin blend was modified slightly from the mean resin composition of all trees, with lower limonene (which is often unstable and usually a deterrent to beetles) and higher myrcene content (table 1). The terpene blend was produced by Chem-Tica Internacional (S.A., Costa Rica).

A mult categorical logit model (PROC CATMOD, SAS 2003) was used to test *Dendroctonus* preferences for the three lure types at 11 locations. The Hualapai site served as a base for comparison for location effect, whereas the odds of preferring myrcene over the blend lure,  $\alpha$ -pinene over blend and  $\alpha$ -pinene over myrcene are response variables in logit model. Lure preferences were significantly different based on the P-value in the analysis of maximum likelihood estimates in the CATMOD procedure.

## Results

### Experiment 1 – comparison of commercial lures and terpene alternatives

A total of 28 271 Coleoptera were captured representing more than 17 species (table 2). *Dendroctonus* bark beetles accounted for 93% of total insects collected. The most abundant *Dendroctonus* species was *D. frontalis* followed by *D. brevicomis*, *D. valens* LeConte, *D. approximatus* Dietz and *D. adjunctus*

Blandford (table 2). The most abundant bark beetle predator was *Temnochila chlorodia* (Mannerheim) (Coleoptera: Trogositidae) followed by *Enoclerus* spp. (Coleoptera: Cleridae). High numbers of *Elacatis* sp. (Coleoptera: Othniidae) and click beetles (Coleoptera: Elateridae) were also captured in traps (table 2). Only *D. frontalis*, *D. brevicomis*, *D. valens*, *T. chlorodia*, *Elacatis* sp., and Elateridae were caught in sufficient numbers to warrant statistical analysis of sampling period and pheromone treatment. Trap catches of *D. frontalis* were relatively uniform throughout the experiment while all other abundant species varied considerably from week to week (table 3). There was a significant Week  $\times$  Lure interaction for *D. brevicomis*, *D. frontalis*, and *T. chlorodia* but not for *D. valens*, *Elacatis* sp. and Elateridae species (table 3). Such interactions probably arose because of weekly fluctuations in captures of these species over the time span of the experiment.

*Dendroctonus brevicomis* and *D. frontalis* showed a significant attraction to the combination of *exo*-brevicomin, frontalin and  $\alpha$ -pinene (fig. 2) over all other lure treatments. The replacement of the myrcene component of the WPB lure with  $\alpha$ -pinene (BFP) attracted less than two times as many beetles of both species. Additionally, lures containing *exo*-brevicomin were significantly more attractive than lures without *exo*-brevicomin. *Dendroctonus valens* demonstrated a clear preference for lures containing terpenes (fig. 2c) while traps with just frontalin or the blank control caught significantly fewer *D. valens*.

**Table 2** Total numbers of Coleoptera collected from Lindgren funnel traps in Arizona during the two trapping experiments

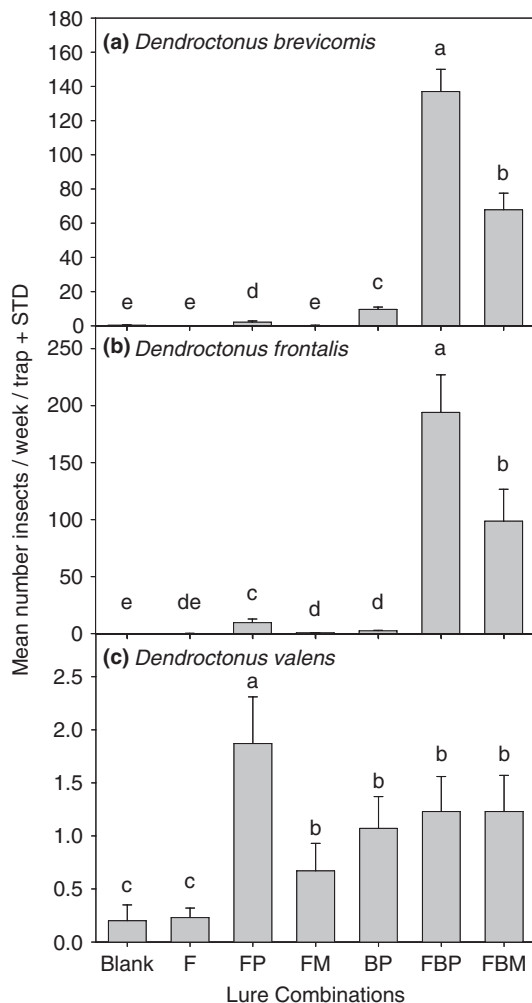
Insect	Family	Guild	Total Experiment 1	Experiment 2
<i>Dendroctonus adjunctus</i>	Scolytinae (Curculionidae)	Herbivores	57	0
<i>Dendroctonus approximatus</i>			60	8
<i>Dendroctonus brevicomis</i>			11 032	11 403
<i>Dendroctonus frontalis</i>			15 241	2745
<i>Dendroctonus valens</i>			290	8
<i>Hylastes</i> spp.			15	1
<i>Ips</i> spp.			145	1
<i>Xyleborus</i> spp.			6	3
<i>Scolytus</i> spp.			3	1
Metallic woodborers	Buprestidae	Herbivores/predators	21	3
Longhorn beetles	Cerambycidae		96	13
<i>Elacatis</i> sp.	Othniidae		189	22
<i>Enoclerus</i> spp.	Cleridae	Predators	81	7
<i>Platysoma</i> spp.	Histeridae		18	1
<i>Temnochila</i> spp.	Trogositidae		683	146
<i>Tenebroides</i> spp.	Trogositidae		59	13
Click beetle spp.	Elateridae	Unknown	278	5

**Table 3** F-values for mixed model anova for the most abundant insects collected in Lindgren funnel traps during the summer 2005 (Experiment 1)

Effect	D.F.	<i>Dendroctonus brevicomis</i>	<i>Dendroctonus frontalis</i>	<i>Dendroctonus valens</i>	<i>Elacatis sp.</i>	<i>Temnochila chlorodia</i>	Elateridae
Week	5	3.97**	0.54	9.31**	6.81**	17.45**	4.97**
Treatment	6	246.8**	142.9**	4.69**	7.00**	24.03**	0.90
Treatment $\times$ week	30	2.17**	2.68**	1.28	1.35	2.20**	0.92

\*\*Indicate P-value &lt; 0.01.

\*\*Indicate P-value &lt; 0.01.

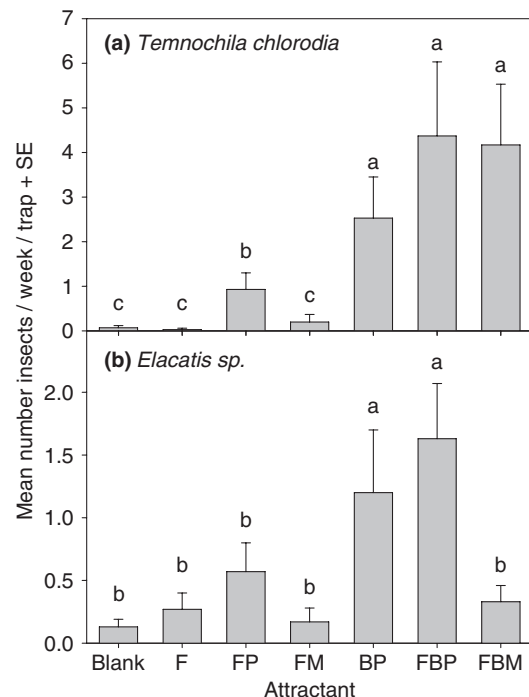


**Fig. 2** Mean number  $\pm$  STD of *Dendroctonus brevicomis* (a), *Dendroctonus frontalis* (b), and *Dendroctonus valens* (c) captured in Lindgren funnel traps baited with various synthetic pheromone lures near Flagstaff, Arizona during the summer 2005 (Experiment 1). Component of lures are: F = frontalin, P =  $\alpha$ -pinene, M = myrcene and B = *exo*-brevicomin.  $n = 10$  blocks  $\times$  six sample periods. Different letters indicate significantly different numbers of insects at  $\alpha = 0.05$ .

Other phloeophagous and wood boring species in the genera *Dendroctonus*, *Ips*, *Hylastes*, *Scolytus* and *Xyleborus* and in the families Buprestidae and

Cerambycidae were collected too infrequently (<1 individual per trap per week) to draw conclusions about their preferences for specific lures.

*Temnochila chlorodia* was the most abundant bark beetle predator (table 2) and was most attracted to lures containing *exo*-brevicomin (fig. 3a). This predator was also attracted to frontalin +  $\alpha$ -pinene, but showed no more attraction to frontalin or frontalin + myrcene relative to its attraction to the blank control. Predators in the families Histeridae and Cleridae were collected too infrequently to draw conclusions.



**Fig. 3** Mean number  $\pm$  SE of *Temnochila* (a) and *Elacatis* (b) captured in Lindgren funnel traps baited with various synthetic pheromone lures near Flagstaff, Arizona during the summer 2005 (Experiment 1). Component of lures are: F = frontalin, P =  $\alpha$ -pinene, M = myrcene, and B = *exo*-brevicomin.  $n = 10$  blocks  $\times$  six sample periods. Different letters indicate significantly different means of each insect collected at  $\alpha = 0.05$ .

Captures of *Elacatis* were strongly influenced by the presence of  $\alpha$ -pinene. *Elacatis* was most attracted to the exo-brevicomin +  $\alpha$ -pinene lure and the frontalin + exo-brevicomin +  $\alpha$ -pinene lure (fig. 2b). The number of *Elacatis* captured in the blank control was not significantly different from the number captured in traps baited with frontalin, frontalin +  $\alpha$ -pinene, frontalin + myrcene, or frontalin + exo-brevicomin + myrcene. There was no significant difference in the number of click beetles (Family: Elateridae) captures across treatments (table 3).

#### Experiment 2 – response to terpenes across Arizona

A total of 14 148 *D. frontalis* and *D. brevicomis* were captured throughout the eight trapping locations (11 sites) in Arizona. The odds of lure preferences, which determined the total trap captures, significantly varied with location (table 4;  $\chi^2 = 986.51$ , d.f. = 20,  $P < 0.001$ ). In nine out of 11 sites, the combination of exo-brevicomin, frontalin and  $\alpha$ -pinene captured more *Dendroctonus* beetles than the combination of exo-brevicomin, frontalin and myrcene or the terpene blend (table 4). Especially at the following sites: Fools Hollow State Park, Kendrick Mt., Tonto N.F. site 1 and two sites at Prescott, the combination of exo-brevicomin, frontalin and  $\alpha$ -pinene captured more than 50% of *Dendroctonus* beetles. However, in two of the locations (Centennial Forest, Mormon Lake), myrcene in combination with exo-brevicomin and frontalin was

the most attractive, and in five locations (Fools Hollow State Park, Apache-Sitgr. sites 1 and 2, Prescott sites 1 and 2) it was significantly less attractive than the other two lures. At Fools Hollow State Park, the lure combination of exo-brevicomin, frontalin and myrcene had almost no *D. frontalis* and *D. brevicomis* tarp captures; whereas at the Mormon Lake site, such lure combinations had approximately 67.5% of captures. At the Hualapai Mountains location (west side of State), only *D. brevicomis* was captured.

Based on the contrasts of maximum likelihood estimates, the lure attraction pattern was significantly different from each other for each pair of sites except for the Apache-Sitgreaves site 2 and Tonto N.F. site 2 (both  $>2100$  m in elevation;  $\chi^2 = 0.86$ ,  $P = 0.65$ ). The lure attraction patterns at two sites in Prescott (both about 1850 m in elevation) were similar ( $\chi^2 = 6.44$ ,  $P = 0.04$ ), which were also the same to that at the Hualapai Mts site (1825 m in elevation;  $\chi^2 \leq 0.86$ ,  $P \geq 0.17$ ). Due to large capture numbers and poor conditions of the insects in many traps, not all *Dendroctonus* could be identified to species and sex, and thus we could not test differences among sexes and species on attraction to each lure.

Relatively low numbers of predators, compared to Experiment 1, were captured in our traps. Most traps caught no predators, with one notable exception; the terpene blend lure at the Prescott 2 site caught 128 *Temnochila* spp., while the other traps at that site had no predator captures.

**Table 4** Relative attractiveness of three lure types (exo-brevicomin + frontalin + myrcene,  $\alpha$ -pinene or a terpene blend) to *Dendroctonus frontalis* and *Dendroctonus brevicomis* in 11 locations across Arizona during the summer 2006 (Experiment 2)

Location	Site no.	Total of <i>Dendroctonus frontalis</i> & <i>Dendroctonus brevicomis</i>	<i>Dendroctonus frontalis</i> (approximately %)	Proportion of <i>Dendroctonus</i> spp. captured per lure WPB w/myrcene WPB w/pinene WPB w/blend		
Apache-Sitgreaves N.F. 1	1	1001	73	0.16 <sup>b</sup>	0.43 <sup>a</sup>	0.41 <sup>b</sup>
Apache-Sitgreaves N.F. 2	2	903	20	0.26 <sup>b</sup>	0.44 <sup>a</sup>	0.30 <sup>b</sup>
Fool Hollow State Park	3	1271	20	0.0 <sup>b</sup>	0.58 <sup>a</sup>	0.42 <sup>a</sup>
Mormon Lake	4	249	66	0.67 <sup>a</sup>	0.12 <sup>c</sup>	0.20 <sup>b</sup>
Tonto N.F. 1	5	3221	17	0.17 <sup>b</sup>	0.68 <sup>a</sup>	0.15 <sup>b</sup>
Tonto N.F. 2	6	1343	19	0.27 <sup>b</sup>	0.42 <sup>a</sup>	0.31 <sup>b</sup>
Centennial Forest	7	84	39	0.46 <sup>a</sup>	0.36 <sup>b</sup>	0.18 <sup>c</sup>
Kendrick Mt.	8	774	18	0.27 <sup>b</sup>	0.55 <sup>a</sup>	0.18 <sup>c</sup>
Prescott N.F. 1	9	1253	10	0.14 <sup>c</sup>	0.52 <sup>a</sup>	0.34 <sup>b</sup>
Prescott N.F. 2	10	3221	10	0.17 <sup>c</sup>	0.51 <sup>a</sup>	0.32 <sup>b</sup>
Hualapai Mts.	11	828	0	0.24 <sup>c</sup>	0.41 <sup>a</sup>	0.36 <sup>b</sup>
TOTAL		14148	27	0.26 <sup>b</sup>	0.46 <sup>a</sup>	0.29 <sup>b</sup>

Letters indicate significant differences in attraction to lures at each location based odds of preferences using maximum likelihood estimates from the PROC CATMOD model, SAS.

## Discussion

The standard commercial lures for attracting *D. brevicomis* and *D. frontalis* were not as effective at capturing beetles and beetle-predators as other pheromone-terpene combinations tested. In contrast to studies in other locations (Bedard et al. 1969, 1980; Vite and Renwick 1971; Payne et al. 1977), the lure that comprised *exo*-brevicomin + frontalin +  $\alpha$ -pinene was most attractive to both *D. frontalis* and *D. brevicomis*. The presence of all three components, frontalin, *exo*-brevicomin and particular terpenes was important variables in determining trap catches of *Dendroctonus* in Arizona, while their predators and associates were primarily influenced by *exo*-brevicomin or  $\alpha$ -pinene and not frontalin.

The increased attraction of *D. frontalis* and *D. brevicomis* to lures with  $\alpha$ -pinene over lures with myrcene suggests that the monoterpene component can significantly affect the attraction and arrestment behaviour of these species. The ternary mixtures of *exo*-brevicomin, frontalin and a terpene were consistently the most attractive to both of these *Dendroctonus* species. Regional differences in attraction of *D. brevicomis* to particular terpenes have been reported several times. For instance, Bedard et al. (1969) and Pitman and Vite (1971) reported that myrcene in combination with *exo*-brevicomin and frontalin was more attractive to *D. brevicomis* than other terpene compounds in California, while Bedard et al. (1980) later found no difference in attraction containing various monoterpenes to *D. brevicomis*. Differences in *D. brevicomis* attraction to pheromone combinations reported in these studies may arise from (i) temporal and spatial differences in beetle attraction to specific terpenes, (ii) differences in experimental methodology, or (iii) differences in stereochemistry, release rates and purity of test compounds. If differences in attraction to lure combinations are representative of what is happening in nature, then the relative effectiveness of commercially available lures probably changes with geographical location. The high attraction of *D. frontalis* to  $\alpha$ -pinene appears universal (Renwick and Vite 1969) and increased release rates of  $\alpha$ -pinene increases beetle attraction. However, inclusion of  $\alpha$ -pinene in a lure did not always make it the most attractive in our study. In two out of 11 sites in Arizona, the combination of *exo*-brevicomin, frontalin and  $\alpha$ -pinene was not as attractive as the combination with the myrcene or the terpene blend. This suggests local adaptation, potentially to host-derived monoterpene composition of trees within area. It is also interesting to note that the two

locations that show greatest attraction to the myrcene lure had the lowest captures of all the sites, implying that none of the lures tested may be very attractive to beetles in these locations. The use of coupled gas chromatographic-electro antennographic detection analysis of different host monoterpenes for these two beetle species may lead to a better understanding of host selection mechanisms, and different optimal lure formulations for trapping.

The finding that *D. frontalis* is attracted to *exo*-brevicomin, in combination with frontalin and a monoterpene, is contrary to earlier studies of the beetle in the southeast United States where *D. brevicomis* does not occur (Payne 1975; Richerson and Payne 1979; Watterson et al. 1982). Payne et al. (1977) found that *exo*-brevicomin reduced or had no effect on *D. frontalis* landing rates within infestations in Texas. However, recent studies by Pureswaran et al. (in press) found that the addition of *exo*-brevicomin to the SPB lure significantly increased trap captures within forests of Mississippi. Our findings are consistent with these findings, and previous studies in Arizona, that demonstrated attraction of *D. frontalis* to *exo*-brevicomin (Sanchez-Martinez and Wagner 2002; Zausen et al. 2005; Gaylord et al. 2006, Williams et al., in press).

Attraction of *D. frontalis* to *exo*-brevicomin may depend upon the proximity of the lure to attacked trees, the release rate of *exo*-brevicomin or the density of beetles within the area. For instance, outside of infestations *D. frontalis* may be attracted to *exo*-brevicomin (Pureswaran et al. in press), but inside infestations *exo*-brevicomin may be disruptive (Payne et al. 1977). Another explanation is that *D. frontalis* is attracted to impurities associated with our *exo*-brevicomin lure. *Endo*-Brevicomin, which is a significant component of male *D. frontalis* pheromone in the southeast United States. (Pitman et al. 1969; Sullivan et al. 2007), may be attractive to *D. frontalis* in the field. However, the quantity of *endo*-brevicomin in the WPB lure is less than 3% of that of *exo*-brevicomin, and is unlikely to significantly attract beetles (B. Sullivan, personal communication). Another explanation is that *D. frontalis* is equally attracted to either stereochemistry of brevicomin, at least outside of beetle infestations as reported here and by Pureswaran et al. (in press). A third explanation is that the attraction to *exo*-brevicomin is a vestigial behavioural trait remaining within this genus (Symonds and Elgar 2004).

The attraction of both *Dendroctonus* species to *exo*-brevicomin in Arizona suggests that *D. frontalis* and *D. brevicomis* may utilize this compound in



conjunction to mass aggregate and overcome host tree defenses. Both species are observed to colonize ponderosa pine in Arizona and co-occur within the same tree (Breece et al. in press). This is contrary to the belief that *D. frontalis* and *D. brevicomis* displace or exclude each other from habitats or areas (Lanier et al. 1988). The advantage each species gains in having cross-attraction of pheromones to mass attack trees (Reid 1999; Ayres et al. 2001) may counterbalance selection for reproductive isolation and the negative effects of interspecific competition (Symonds and Elgar 2004). Mate recognition and reproductive isolation between these species may result from differences in their pheromone composition that is not represented in the commercial lures (Byers 1989). Pheromone analyses of *D. frontalis* and *D. brevicomis* collected in Arizona, reveal slight differences in pheromone components between species that could account for reproductive isolation (Pureswaran et al. in press). However, field trials with infested logs by each individual beetle species, reveals that both species are most attracted to logs infested with *D. brevicomis* females (Davis and Hofstetter, unpublished). Reproductive isolation may also occur through differences in short-range airborne or vibrational communication once beetles have landed on the tree (Rudinsky 1973; Rudinsky et al. 1974). Comparison of acoustic specificity in sympatric and allopatric bark beetles may show reproductive character displacement not found in olfactory signals (Symonds and Elgar 2004). Behavioral mechanisms for avoiding interspecific competition likely occur between these species but are yet unknown.

Responses to terpene-beetle pheromone combinations varied with non-bark beetle species. The kairomone response of the predator, *T. chlorodia*, appears rather specialized for *D. brevicomis* (Bedard et al. 1969; Pitman and Vite 1971), in that it responds strongly to *exo*-brevicomin and less so to frontalin. *exo*-Brevicomin is released by other *Dendroctonus* species that occur in Arizona (Symonds and Elgar 2004; Pureswaran et al. (in press)) and *T. chlorodia* may use this compound to successfully locate a diverse prey base. For *D. frontalis*, the co-attraction to *D. brevicomis* pheromones (i.e. *exo*-brevicomin) to successfully overcome tree defenses may outweigh potential losses due to predation by *T. chlorodia* or interspecific competition for space. Too few *Enoclerus* predators were caught during our experiments to make statistical inferences on kairomonal responses. However, previous studies have shown attraction of *Enoclerus* to lures con-

taining ipsdienol (Miller and Borden 1990) and *trans*-verbenol (Schmitz 1978). The beetle *Elacatis* was strongly influenced by the terpene component, with lure combinations containing  $\alpha$ -pinene being most attractive. Tree preference and feeding behaviour of *Elacatis* is not well known. It was previously believed to be a generalist predator of both primary and secondary bark beetles (Gaylord et al. 2006), but may actually feed on decayed tree tissues based on examination of larval gut contents (D. Pollock, pers. comm.). Its attraction to volatile cues from host trees under beetle attack may allow it to locate a tree early during tree decay.

### Implications for management

The  $\alpha$ -pinene + *exo*-brevicomin + frontalin lure allows for greater trapping of *D. frontalis* and *D. brevicomis* in Arizona, and would improve monitoring of beetle populations at landscape level. *Temnochila*, the prominent *Dendroctonus* predator in the region (Gaylord et al. 2006), was no more attracted to the  $\alpha$ -pinene lures and thus such lures are potentially good candidates for *Dendroctonus* control. Further studies are needed to determine optimal release rates for developing a lure most appropriate for Arizona.

### Acknowledgements

We thank Elisabeth Alden, Gabe DeGomez, Kevin Johnson, Laine Smith, and James Stewart for their assistance in collecting and counting insects. We also thank Fool Hollow State Park, Apache/Sitgreaves National Forest (NF), Coronado NF, Coconino NF, Prescott NF and Tonto NF for trap collections. We thank two anonymous reviewers. This project was funded by Western Bark Beetle Initiative U.S.D.A. 05-PA-11221615-161, U.S.D.A. Cooperative Agreement 06-CA-11330129-046, and supported by School of Forestry Northern Arizona University and U.S.D.A. Forest Service.

### References

- Aukema BH, Raffa KF, 2005. Selective manipulation of predators using pheromones: responses to frontalin and ipsdienol pheromone components of bark beetles in the Great Lakes region. *Agric. Forest Entomol.* 7, 193–200.
- Aukema BH, Dahlsten DL, Raffa KF, 2000. Exploiting behavioral disparities among predators and prey to selectively remove pests: maximizing the ratio of bark beetles to predators removed during semiochemically based trap-out. *Environ. Entomol.* 29, 651–660.

- Ayres BD, Ayres MP, Abrahamson MD, Teale SA, 2001. Resource partitioning and overlap in three sympatric species of *Ips* bark beetles (Coleoptera: Scolytidae). *Oecologia* 128, 443–453.
- Bedard WD, Tilden PE, Wood DL, Silverstein RM, Brownlee RG, Rodin JO, 1969. Western pine beetle: field response to its sex pheromone and a synergistic host terpene, myrcene. *Science* 164, 1284–1285.
- Bedard WD, Wood DL, Tilden PE, Lindahl KQ Jr, Silverstein RM, Rodin JO, 1980. Field response of the western pine beetle and one of its predators to host- and beetle-produced compounds. *J. Chem. Ecol.* 6, 625–641.
- Berisford CW, Payne TL, Berisford Y, 1990. Geographic variation in response of southern pine beetle (Coleoptera: Scolytidae) to aggregating pheromones in laboratory bioassays. *Environ. Ent.* 19, 1671–1674.
- Billings RF, Bryant CM, 1983. Developing a system for mapping the abundance and distribution of southern pine beetle habitats in east Texas. *Z. Angew. Entomol.* 96, 208–216.
- Borden JmJinHuBryantMP,M[(erettle)-329R0(RG,)-33Sbeesortles

- Reid ML, 1999. Monogamy in the bark beetle *Ips latidens*: ecological correlates of an unusual mating system. *Ecol. Entomol.* 24, 89–94.
- Renwick JAA, Vite JP, 1969. Bark beetle attractants: mechanisms of colonization by *Dendroctonus frontalis*. *Nature* 244, 1222–1223.
- Richerson JV, Payne TL, 1979. Effects of bark beetle inhibitors on landing and attack behavior of the southern pine beetle and beetle associates. *Environ. Entomol.* 8, 360–364.
- Rudinsky JA, 1973. Multiple functions of the southern pine beetle pheromone verbenone. *Environ. Entomol.* 2, 511–514.
- Rudinsky JA, Morgan ME, Libbey LM, Putman TB, 1974. Anti-aggregation – rivalry pheromone of the mountain pine beetle and new arrestant of the southern pine beetle. *Environ. Entomol.* 3, 90–98.
- Sanchez-Martinez G, Wagner MJ, 2002. Bark beetle community structure under four ponderosa pine forest stand conditions in northern Arizona. *For. Ecol. Manage.* 170, 145–160.
- SAS, 2003. The SAS system for windows, version 9.1, SAS Institute Inc., Cary, NC 27513.
- Schmitz RF, 1978. Taxonomy and bionomics of the North American species of *Thanasimus* Latreille (Coleoptera: Cleridae). Ph.D. dissertation. University of Idaho, Moscow.
- Seybold SJ, Huber DP, Lee JC, Graves AD, Bohlmann J, 2006. Pine monoterpenes and pine bark beetles: a marriage of convenience for defense and chemical communication. *Phytochem. Rev.* 5, 143–178.
- Skillen EL, Berisford CW, Camann MA, Reardon RC, 1997. Semiochemicals of forest and shade tree insects in North America and management implications. USDA Forest Service FHTET-96-15, 184.
- Smith RH, 1975. Formula for describing effect of insect and host tree factors on resistance to western pine beetle attack. *J. Econ. Entomol.* 68, 841–844.
- Smith RH, 2000. Xylem monoterpenes of pines: distribution, variation, genetics, function. Gen. Tech. Report PSW-GTR-177, 454.
- Smith MT, Payne TL, Birch MC, 1990. Olfactory-based behavioral interactions among five species in the southern pine bark beetle group. *J. Chem. Ecol.* 16, 3317–3332.
- Strom BL, Goyer RA, Ingram LL Jr, Boyd GDL, Lott LH, 2002. Oleoresin characteristics of progeny of loblolly pines that escaped attack by the southern pine beetle. *For. Ecol. Manage.* 158, 169–178.
- Sturgeon KB, 1979. Monoterpene variation in ponderosa pine xylem resin related to western pine beetle predation. *Evolution* 33, 803–814.
- Sullivan BT, Shepherd WP, Pureswaran DS, Mori K, 2007. Evidence that (+)-endo-brevicomin is a male produced aggregation pheromone component of the southern pine beetle, *Dendroctonus frontalis*. *J. Chem. Ecol.* 33, 1510–1527.
- Svihra P, Paine TD, Birch MC, 1980. Interspecific olfactory communications in southern pine beetle. *Naturwissenschaften* 67, 518.
- Symonds MRE, Elgar MA, 2004. Species overlap, speciation and the evolution of aggregation pheromones in bark beetles. *Ecol. Lett.* 7, 202–212.
- USDA Forest Service, 2001–2006. Forest insect and disease conditions in the Southwestern Region. USDA Forest Service, Southwestern Region, Forestry and Forest Health, Albuquerque, NM. <http://www.fs.fed.us/r3/publications/>
- Vite JP, Renwick JAA, 1971. Inhibition of *Dendroctonus frontalis* response to frontalin by isomers of brevicomin. *Naturwissenschaften* 58, 418.
- Watterson GP, Payne TL, Richerson JJ, 1982. The effects of verbenone and endo-brevicomin on the within tree populations of *Dendroctonus frontalis*. *J. Geo. Entomol. Soc.* 17, 118–126.
- Williams KK, McMillin JD, DeGomez TE, Clancy KM, Miller A. Influence of elevation on bark beetle (Coleoptera: Curculionidae, Scolytinae) community structure and flight periodicity in Ponderosa pine forests of Arizona. *Environ. Entomol.* (in press).
- Wood DL, 1973. New synonymy in American bark beetles (Scolytidae: Coleoptera), Part III. *Great Basin Nat.* 33, 169–188.
- Wood DL, 1982a. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. *Ann. Rev. Entomol.* 27, 411–446.
- Wood SL, 1982b. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. *Great Basin Naturalist Memoirs* No. 6, Brigham Young University, Provo, Utah.
- Zausen GL, Kolb TE, Bailey JD, Wagner MR, 2005. Long-term impacts of stand management on ponderosa pine physiology and bark beetle abundance in northern Arizona: a replicated landscape study. *For. Ecol. Manage.* 218, 291–305.